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Comparative Effects of the Sorghum *bmr*-6 and *bmr*-12 Genes: I. Forage Sorghum Yield and Quality

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ABSTRACT

Brown midrib (bmr) forages usually contain less lignin and exhibit increased digestibility. Recent research has identified the modifications in biochemical pathways resulting from bmr mutations. In sorghum [*Sorghum bicolor* (L) Moench.], *bmr*-6 has been linked to a decrease in cinnamyl alcohol dehydrogenase (CAD) activity. The allelic *bmr*-12 and *bmr*-18 genes decrease caffeic acid *O*-methyl transferase (OMT) activity. There has been only limited research comparing bmr genes to each other and wild type in isogenic sorghum. The objective of this study was to determine the impact of *bmr*-6 and *bmr*-12 on forage yield and quality in these genetic backgrounds: 'Atlas', 'Early Hegari-Sart', 'Kansas Collier', and 'Rox Orange'. Height, lodging, neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), in vitro NDF digestibility (IVNDFD), and dry matter (DM) yield were measured in a split-plot experiment replicated four times in each of four environments with lines being whole-plots and genotypes (wild type, *bmr*-6, and *bmr*-12) being subplots. Brown midrib genes generally had negative agronomic impact, but these were not uniformly expressed across backgrounds. The *bmr*-6 gene generally resulted in a shorter plant and less DM yield, but did not reduce ADL. The *bmr*-12 gene generally resulted in reduced ADL, later maturity, and reduced or equivalent DM yield when compared with the wild type. There is a more digestible NDF fraction in both *bmr*-6 and *bmr*-12 forage sorghums. When all data are considered in aggregate, the *bmr*-12 gene appears superior to the *bmr*-6 gene in terms of less negative impact on agronomic performance and greater positive impact on ADL content and fiber digestibility.

Forage sorghum is an important annual forage source in the midwestern and plains regions of the USA and can be planted later than maize (*Zea mays* L.). It uses water more efficiently, yields greater biomass, and provides an acceptable yield when exposed to drought (Sanderson et al., 1992). However, maize hybrids typically have greater dry matter digestibility than forage sorghums. Lignin, found in plant cell walls, is the second most abundant polymer in nature after cellulose (Jung and Ni, 1998), and while being beneficial to plants, the complex linkages in lignin are detrimental to the digestibility of plant cell walls by livestock (Humphreys and Chapple, 2002). The lignin content of the whole maize plant is less than that of typically fed forage sorghums.

Chemical and genetic approaches have been employed to improve forage fiber digestibility by reducing the amount of lignin or the extent of lignin cross linking with cell wall carbohydrates. Brown midrib forage genotypes usually contain less lignin and have altered lignin chemical composition (Bucholtz et al., 1980; Cherney et al., 1991; Vogel and Jung, 2001). To date, genetic control of the lignification process through manipulation of the bmr trait has offered the most direct and productive approach to reducing lignin content and increasing digestibility of forage sorghums (Gerhardt et al., 1994). Brown midrib mutants of maize have been known for nearly four decades (Jorgenson, 1931) and since then the mutation has been observed in pearl millet [*Pennisetum americanum* (L.) Leeke] (Cherney et al., 1988), sorghum (Porter et al., 1978), and sudangrass [*Sorghum* × *drummondii* (Steud.) Millsp. & Chase] (Fritz et al., 1981). In situ and in vitro digestion studies have shown bmr forages to have greater digestion than their normal counterparts.

Brown midrib mutations in sorghum were induced by Porter et al. (1975) by soaking sorghum seeds in diethyl sulfate. This resulted in 19 bmr mutant lines. Of these, three were selected as most agronomically acceptable (Fritz et al., 1988) and form the basis for considerable additional research and line development using the three bmr sorghum genes *bmr*-6, *bmr*-12, and *bmr*-18. Further examination has yielded information indicating *bmr*-12 and *bmr*-18 genes are allelic (Bittinger et al., 1981), and that the *bmr*-6 and the *bmr*-12 and *bmr*-18 genes are located at two independent loci (Gupta, 1995).

Recent research has identified modifications in biochemical pathways which result from the bmr mutations. Two separate enzymes exhibit reduced activity as the result of bmr mutations. In sorghum, the *bmr*-6 gene has been linked to a decrease in lignin due to a decrease in CAD activity (Bucholtz et al., 1980). The allelic *bmr*-12 and *bmr*-18 genes decrease caffeic acid OMT activity (Bout and Vermerris, 2003), specifically, via a premature stop condon. Due to these differences in lignin synthesis, many previous studies have investigated the impacts these individual genes have on the resulting animal production response. Forages containing bmr genes have increased digestibility when compared to wild-type lines (Akin et al., 1986a; Grant et al., 1995; Wedig et al.,

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Abbreviations: ADF, acid detergent fiber; ADL, acid detergent lignin; bmr, brown midrib; CAD, cinnamyl alcohol dehydrogenase; DM, dry matter; IVNDFD, in vitro neutral detergent fiber digestibility; NDF, neutral detergent fiber; OMT, O-methyl transferase.

1987). Brown midrib lines of corn have improved NDF digestibility in vitro (Greenfield et al., 2001), greater apparent DM, ADF, and organic matter digestibility when compared to isogenic wild-type lines (Tine et al., 2001). Previous research (Aydin et al., 1999) observed greater milk production for Holstein dairy cows fed bmr sorghum versus wild-type forage sorghum, with milk production similar to cows fed corn silage.

However, considerable variation in forage quality parameters, including NDF, ADF, and digestibility, has also been shown to exist among different wild-type (not bmr) sorghum lines (Lema et al., 2000) which could confound comparisons among bmr genes. There has been only very limited research comparing individual bmr genes to isogenic wild-type sorghum (Hanna et al., 1981; Thorstensson et al., 1992), and even less research comparing bmr genes to each other in similar or isogenic backgrounds.

Our previous research compared the effect of two forage sorghum hybrids, one with *bmr*-6 and one with *bmr*-18, and found bmr hybrids did not elicit the same production response when fed to lactating dairy cattle (Oliver et al., 2004). However it was not possible to separate the effect of bmr genes from hybrid in that study. To date, there has been no comparison among the bmr genes to determine if one of the genes is truly superior in an array of forage sorghum backgrounds. Therefore, the objective of this study was to determine the impact of the *bmr*-6 and *bmr*-12 genes on forage yield and quality in a broad set of forage sorghum lines.

MATERIALS AND METHODS

Near-isogenic versions of four forage sorghum lines (Atlas, Early Hegari-Sart, Kansas Collier, and Rox Orange) were created by crossing each to N121 (Gorz et al., 1990), a *bmr*-6 source, and F220 or F324 (donated to our project by the late Robert Kalton), *bmr*-12 sources, followed by four backcrosses to the recurrent parents with selection for the bmr phenotype. Field trials using the recurrent parents and their counterparts near-isogenic for *bmr*-6 and *bmr*-12 were conducted in 2002 and 2003 at the University of Nebraska Field Laboratory, Ithaca, NE, (Sharpsburg silty clay loam; fine, smectitic, mesic Typic Argiudoll) and Lincoln, NE [Kennebec silt loam (fine-silty, mixed, superactive, mesic Cumulic Hapludoll)]. Individual plots consisted of three 7.6-m rows spaced 76 cm apart. Each plot was seeded with a precision vacuum planter calibrated to deliver 120 seeds per row (240 000 seeds ha^{-1}). The near-isogenic sets (e.g., Atlas, Atlas *bmr*-6, Atlas *bmr*-12) were planted as a block to minimize border effects.

The experiments were planted 20 May 2002 and 21 May 2003 in Lincoln and 22 May 2002 and 2003 in Ithaca. Nitrogen fertilizer was applied preplant at both locations at 157 kg ha^{-1}. At the Lincoln location, propachlor [2-chloro-*N*-(1-methylethyl)-*N*-phenylacetamide] and atrazine [6-chloro-*n*-ethyl-*N*′-(1-methylethyl)-1,3,5-triazine-2,4,diamine], applied at 3.36 and 1.1 kg ha^{-1}, respectively, were applied immediately after planting for weed control. No supplemental irrigation was applied at Lincoln. At the Ithaca location, atrazine was applied at 2.2 kg ha^{-1} immediately after planting, followed by an application of quinclorac (3,7-dichloro-8-quinolinecarboxylic acid) and atrazine at 0.37 kg ha^{-1} and 1.1 kg ha^{-1}, respectively approximately 14 d post-emergence. In 2002, bentazon [3-(1-methylethyl)-1*H*-2,1,3-benzothiadiazin 4(3*H*)-one-2,2-dioxide] was added to the post-emergence application at 0.28 kg ha^{-1} for velvetleaf [*Abutilon theophrasti* (Medik)] control. Grasshoppers [*Dissosteira Carolina* (Linnaeus)] were controlled by application of chloropyrifos [phosphorodithioic acid, *O,O*-diethyl *O*-(3,5,6-trichloro-2-pyridyl) ester] on 18 and 24 July 2002 and 17 July 2003. Five centimeters of supplemental irrigation was applied at Ithaca via overhead sprinklers on 24, 28 June and 5, 7 August in 2002. In 2003, 2.5 cm of supplemental irrigation was applied on 24 July and 14 and 28 August, and 5 cm of supplemental irrigation was applied on 4 and 7 August.

Days to flowering was recorded at 50% anthesis. Percentage of plants lodged in each plot was estimated visually immediately before harvest. Plots were harvested using a commercial silage cutter modified for small plot use (Pedersen and Moore, 1995). The Lincoln location was harvested 5 September 2002 and 29 September 2003. Ithaca plots were harvested 11 September 2002 and 25 September 2003. All plots were at hard dough or were fully mature. Plot wet weights were recorded and subsamples collected from the middle row of each plot for moisture and forage quality analyses.

Sample Analysis

Subsamples were oven dried at 60°C, dry weights recorded, and ground through a Wiley mill (Arthur H. Thomas Co., Philadelphia, PA) to pass a 1-mm screen. Each sample was analyzed sequentially for NDF, ADF, and ADL using an ANKOM 200 fiber analyzer (ANKOM Tech. Corp., Fairport, NY) (Vogel et al., 1999). Ground sample was weighed into ANKOM F57 filter bags (ANKOM Tech. Corp., Fairport, NY), 0.5 ± 0.0025 g, and the bags were heat sealed. The samples were then suspended in 1900 mL of NDF detergent (Midland Scientific Inc., Omaha, NE) with 3 mL of heat stable α-amylase (ANKOM Tech. Corp., Fairport, NY). Once the temperature reached 95°C, samples were agitated for 60 min. The heat and agitation were then stopped and the NDF solution was drained from the fiber analyzer and 2 L of 95°C distilled water and 3 mL of heat stable α-amylase where added, agitated for 5 min, and drained. Three rinses of 3 min with 2 L of 95°C distilled water followed. Samples were transferred to a wire basket, rinsed with 25°C distilled water, and dried for 12 h in a 100°C oven. Following weigh back, samples were refluxed in ADF solution (Midland Scientific Inc., Omaha, NE) for 60 min at 95°C. The samples were rinsed for 5 min with 95°C water and then four more rinses of 3 min each were done. Samples were again dried in a wire basket for 12 h in a 100°C oven and weighed. ADL was performed by submerging 25 samples in 333 mL of 72% sulfuric acid for 3 h. Samples were stirred every 30 min to ensure uniform dispersion of the acid. Samples were rinsed with 95°C distilled water and 25°C distilled water until the rinse water reached a neutral pH. The samples were then dried in a wire basket for 12 h in a 100°C oven.

In vitro NDF digestion was performed using ANKOM rumen fermenters (Model No: Daisy II; ANKOM Tech. Corp., Fairport, NY). ANKOM F57 filter bags (25-μm pore size) of 0.55 ± 0.0025 g of sample were heat sealed and incubated for 30 h in 1600 mL of rumen inoculum and 400 mL of rumen buffer at a pH of 6.8 (Goering and Van Soest, 1970). Rumen inoculum was collected from a steer being fed a mixed diet 12 h post-feeding. Thirty-six bags were incubated in each glass jar and were purged with CO_2 before being placed in the incubator. After 30 h, samples were removed and frozen. NDF analysis was then preformed on the digested sample following the same procedures described previously.

Statistical Analysis

Experimental design was a split-plot replicated four times in each of four environments with lines being whole-plots and

genotypes (wild type, *bmr*-6, and *bmr*-12) being subplots. The data were analyzed using the PROC MIXED procedure of SAS (SAS Institute, Inc., 1999). The model statement included line, gene, and the line × gene effects. Environments and replication were considered random. The REPEATED function of PROC MIXED was used to account for lack of homogeniety of variance among the environments. *F*-protected least significant differences were used to determine differences among lines and genes.

RESULTS AND DISCUSSION

In side-by-side plots, the midribs of the *bmr*-6 near-isolines exhibited more intense brown coloration than the *bmr*-12 near-isolines (Fig. 1). There was a significant effect of the environment on the measured traits as expected. Since our objective was to determine the effects of the bmr genes across multiple environments and multiple genetic backgrounds, we accounted for environment, line, and environment interactions in our model and report the pooled gene and gene × line means.

The *bmr*-12 near-isolines reached 50% anthesis an average of 3 d later than *bmr*-6 and 4 d later than wild-type near-isolines in forage sorghum (Table 1). There were significant gene effects and line × gene interactions for days to 50% anthesis. The *bmr*-12 near-isolines reached anthesis later than either the wild-type or *bmr*-6 near-isolines of Atlas, Early Hegari-Sart, and Rox Orange, and days to 50% anthesis was equivalent in Atlas *bmr*-6 and *bmr*-12 near-isolines. The effect of *bmr*-6 compared to wild type was inconsistent, with days to 50% anthesis being equivalent in Rox Orange, greater for *bmr*-6 in Early Hegari-Sart and Kansas Collier, and greater for wild type in Atlas. Previous research has found flowering time to differ in bmr plants when compared with wild types. Vermerris et al. (2002) found *bmr*-6 to flower later than the wild type while *bmr*-18 (allelic to *bmr*-12) flowered at the same time as the wild type. The different flowering response due to line × gene interaction observed in our study clearly demonstrates the importance of genetic background on bmr gene expression.

Height was significantly affected by bmr genes. Line × gene interactions were not significant. In these four forage sorghums, wild-type lines were generally taller than their *bmr*-12 near-isolines, which were taller than their *bmr*-6 near-isolines. Previous studies indicated that when height was measured at the time of flowering *bmr*-6 plants were shorter than the wild type (Anterola and Lewis, 2002). The current study confirmed those results with all *bmr*-6 near-isolines being shorter than their wild-type counterparts at maturity.

There were significant gene effects and line × gene interactions for yield. At harvest the wild type generally yielded the most DM. Averaged over lines, *bmr*-12 near-isolines yielded 10% less than the wild-type near-isolines and *bmr*-6 near-isolines yielded 15% less than the wild-type near-isolines. The wild-type near-isolines had greater DM yield than *bmr*-6 near-isolines in all lines except Rox Orange. In the Early Hegari-Sart background, there was no difference between the wild-type and *bmr*-12 near-isolines for DM yield. Previous research has found decreased yield associated with bmr maize (Miller et al., 1983). Yield differences due to bmr genes in sorghum have not been reported previously, so the impact on yield was assumed to be similar to that of maize (Kalton, 1988). The current study indicates bmr genes do generally elicit a similar yield response in both maize and forage sorghum. However in some genetic backgrounds, yields of either *bmr*-6 or *bmr*-12 near-isolines were equivalent to their wild-type counterparts indicating that yield reduction associated with bmr genes is not absolute in forage sorghum.

Differences in observed lodging were not attributable to bmr genes. Line had a significant impact on the percentage lodging, but there was no line × gene interaction.

Stalks of maize which express a bmr gene have a 17

Fig. 1. Rox Orange *bmr*-6 (left) and Rox Orange *bmr*-12 (right).

to 26% decrease in the crushing strength of the stalk (Zuber et al., 1977). Therefore, lodging problems are typically associated with the inclusion of bmr genes. Minor lodging did occur in our forage sorghum plots but averaged only 23% lodged plants. Previous research (Bean et al., 2002) reported bmr sorghum to be up to 87% lodged. Lodging is greatly dependent on the environmental conditions, with conditions such as rainfall and wind having considerable impact on lodging. Anecdotal observations from producers, and during seed increase of the near-isolines used in this study, indicate increased risk of lodging in bmr forage sorghums when compared to wild-type forage sorghums.

Forage Composition

The fiber analysis of the bmr near-isolines showed that bmr genes had no affect on NDF content of forage sorghum. Line effects were significant, but line × gene were not significant. These findings agree with previous research (Aydin et al., 1999; Grant et al., 1995) in which *bmr*-18 forage sorghum NDF content did not differ from a wild-type forage sorghum (non-isogenic lines). Thorstensson et al. (1992) observed an increase in NDF content of conventional sorghum when compared to *bmr*-6. However, the same study did not detect a difference between a *bmr*-18 line and its normal counterpart.

A strongly significant line × gene interaction was detected for ADF content. Wild-type Atlas had greater ADF than either bmr near-isolines. No differences in ADF attributable to bmr genes were observed in Kansas Collier or Rox Orange. In Early Hegari-Sart, the *bmr*-12 near-isoline was equivalent to wild type and higher than the *bmr*-6 near isoline for ADF content. Cherney et al. (1991) summarized sorghum fiber research and concluded there is considerable variation in fiber composition among conventional and bmr sorghum hybrids. The current study confirms the importance of the line × gene interaction in contributing to the considerable variation in fiber composition among forage sorghum lines.

Gene effects were significant for ADL content of

Table 1. Average and individual effect of *bmr* genes on agronomic and forage quality traits in four forage sorghum lines.

		Wild type	*bmr*-6	*bmr*-12	SEM
Days to 50% anthesis (d)					
	Mean	**74 b†**	**75 b**	**78 a**	**2**
	Atlas	**80 b**	**77 c**	**82 a**	**2**
	Early Hegari-Sart	**71 c**	**74 b**	**78 a**	**2**
	Kansas Collier	**76 b**	**77 a**	**77 a**	**2**
	Rox Orange	**71 b**	**70 b**	**74 a**	**2**
Height (cm)					
	Mean	**215 a**	**194 c**	**211 b**	**12**
	Atlas	**238 a**	**216 c**	**230 b**	**12**
	Early Hegari-Sart	**153 a**	**139 b**	**156 a**	**12**
	Kansas Collier	**236 a**	**214 c**	**228 b**	**12**
	Rox Orange	**232 a**	**205 b**	**230 a**	**12**
Lodging (%)					
	Mean	**23**	**23**	**22**	**8**
	Atlas	**36**	**36**	**36**	**9**
	Early Hegari-Sart	**7**	**7**	**7**	**9**
	Kansas Collier	**18**	**19**	**18**	**9**
	Rox Orange	**30**	**29**	**29**	**9**
DM‡ yield (t ha^{-1})					
	Mean	**15.0 a**	**12.8 c**	**13.5 b**	**1.0**
	Atlas	**15.9 a**	**14.5 b**	**13.6 c**	**1.1**
	Early Hegari-Sart	**13.4 a**	**10.1 b**	**13.8 a**	**1.1**
	Kansas Collier	**15.4 a**	**11.7 c**	**14.5 b**	**1.1**
	Rox Orange	**15.4 a**	**14.8 a**	**12.0 b**	**1.1**
NDF (g kg^{-1})					
	Mean	**454**	**449**	**463**	**12**
	Atlas	**484**	**460**	**475**	**14**
	Early Hegari-Sart	**458**	**433**	**457**	**14**
	Kansas Collier	**437**	**468**	**463**	**14**
	Rox Orange	**436**	**434**	**458**	**14**
ADF (g kg^{-1})					
	Mean	**269 a**	**262 b**	**268 a**	**11**
	Atlas	**305 a**	**286 b**	**284 b**	**12**
	Early Hegari-Sart	**263 ab**	**256 b**	**270 a**	**12**
	Kansas Collier	**260**	**273**	**258**	**12**
	Rox Orange	**246**	**233**	**262**	**12**
ADL (g kg^{-1})					
	Mean	**70 a**	**67 a**	**61 b**	**10**
	Atlas	**75 a**	**72 ab**	**65 b**	**10**
	Early Hegari-Sart	**74 a**	**76 a**	**61 b**	**10**
	Kansas Collier	**65**	**62**	**62**	**10**
	Rox Orange	**66 a**	**57 b**	**55 b**	**10**
IVNDFD (g kg^{-1})					
	Mean	**646 b**	**666 a**	**655 a**	**9**
	Atlas	**604 b**	**646 a**	**630 a**	**11**
	Early Hegari-Sart	**651**	**662**	**655**	**11**
	Kansas Collier	**658**	**658**	**663**	**11**
	Rox Orange	**671 b**	**696 a**	**672 b**	**11**

† Means in rows with differing superscripts differ at $P = 0.05$ using an *F*-protected LSD.
‡ DM, dry matter; NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin; IVNDFD, 30-h in vitro NDF digestibility.

mature forage sorghum, and there was no line × gene interaction. The *bmr*-12 gene resulted in the least amount of ADL. There was no difference in the ADL content of wild-type and *bmr*-6 near-isolines. Previous research indicates both *bmr*-6 and *bmr*-18 forage sorghum have decreased ADL when compared to wild-type forage sorghum silage (Gerhardt et al., 1994; Grant et al., 1995; Lam et al., 1996; Thorstensson et al., 1992). The current study confirms that *bmr*-12 forage sorghum near-isolines have significantly less ADL than their wild-type near-isogenic counterparts, and unexpectedly demonstrates that *bmr*-12 forage sorghum near-isolines have significantly less ADL than near-isogenic *bmr*-6 counterparts across an array of forage sorghum lines.

Gene effects were significant for IVNDFD, and there was no line × gene interaction. The *bmr*-6 and *bmr*-12 near-isolines generally had higher average IVNDFD than wild-type counterparts. The increased IVNDFD associated with the *bmr*-12 near-isolines was expected based on the reduced ADL associated with that gene and is fully supported by numerous reports that bmr mutants have increased IVDMD when compared with normal forage sorghum (Akin et al., 1986b; Cherney et al., 1986; Fritz et al., 1988; Porter et al., 1978). A previous report (Aydin et al., 1999) observed a significant improvement in NDF digestibility of bmr sorghum when compared to a conventional sorghum hybrid. Similarly, Thorstensson et al. (1992) found potentially digestible NDF to be 19% greater in brown midrib mutants.

The increased IVNDFD compared to wild-type near isolines associated with *bmr*-6 is consistent with previous bmr literature but is not consistent with the observed ADL content of the wild-type forage sorghum lines used in this study and their *bmr*-6 near-isoline counterparts. One possibility for this discrepancy may lie with potentially differing lignin chemistry because of reduced OMT (vs. CAD) activity and our choice of using ADL to measure lignin content. Acid detergent lignin accounts for the core lignin while many studies report permanganate lignin (PML) which includes non-core lignin (Van Soest et al., 1991). We reported in a previous study (Oliver et al., 2004) that a *bmr*-18 forage sorghum had greater ADL than a non-isogenic *bmr*-6 forage sorghum hybrid. The same study evaluated permanganate lignin content and the two bmr hybrids were reversed in ranking of their lignin concentration. Fukushima and Dehority (2000) found lignin content of seven different forages to differ significantly among the ADL and PML procedures. Regardless of the method used, the amount of lignin in a forage sample has an impact on the digestibility of the forage (Traxler et al., 1998).

In conclusion, the addition of bmr genes generally has negative agronomic impact on forage sorghum lines, but these negative impacts are not uniformly expressed across multiple genetic backgrounds. The *bmr*-6 gene generally results in a shorter plant and less DM yield per unit land area. The *bmr*-12 gene generally results in later maturity, and reduced or equivalent DM yield when compared with the wild type. Evaluation of specific forage sorghum lines × bmr gene combinations in which the positive nutritive effects of the bmr genes can be combined with acceptable agronomic performance is needed.

There is a more digestible NDF fraction in both *bmr*-6 and *bmr*-12 forage sorghums, corresponding to a greater digestible DM. When all data are considered in aggregate, the *bmr*-12 gene appears superior to the *bmr*-6 gene in terms of less negative impact on agronomic performance and greater positive impact on ADL content and fiber digestibility.

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